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Oral aspects in celiac disease children: clinical and dental enamel chemical evaluation

Fabrício Kitazono de Carvalho, PhD,[a] Alexandra Mussolino de Queiroz, PhD,[b] Raquel Assed Bezerra da Silva, PhD,[c] Regina Sawamura, PhD,[d] Luciano Bachmann, PhD,[e] Léa Assed Bezerra da Silva, PhD,[f] and Paulo Nelson-Filho, PhD[g]

Objective. The aim of this study was to evaluate the oral manifestations of celiac disease (CD), the chemical composition of dental enamel, and the occurrence of CD in children with dental enamel defects (DEDs).

Study Design. In the study, 52 children with CD and 52 controls were examined for DEDs, recurrent aphthous stomatitis (RAS), dental caries experience, and salivary parameters. In addition, 10 exfoliated primary enamel molars from each group were analyzed by energy dispersive x-ray spectroscopy and Fourier transform infrared spectroscopy. Fifty children with DEDs were submitted to CD diagnosis.

Results. Among the children with CD, a higher prevalence of DEDs ($P = .00001$) and RAS ($P = .0052$), lower caries experience ($P = .0024$), and reduction of salivary flow ($P = .0060$) were observed. Dental enamel from the children with CD demonstrated a lower calcium-to-phosphorus ratio ($P = .0136$), but no difference in the carbonate-to-phosphate ratio ($P = .5862$) was observed. In the multivariate analysis, CD was a protective factor for caries (OR = 0.74) and a risk factor for RAS (OR3.23).

Conclusions. The children with CD presented with more RAS, DEDs, reduction of salivary flow, and chemical alterations in the enamel. (Oral Surg Oral Med Oral Pathol Oral Radiol 2015;119:636-643)

Celiac disease (CD) is a chronic, immune-mediated disease of the small intestine caused by exposure to dietary gluten in genetically predisposed individuals.[1,2] The worldwide mean prevalence of CD is 1%, and it is one of the most frequent types of food intolerance.[1-3] There are asymptomatic individuals who may remain undiagnosed despite the advances in the detection of this condition. Clinically, CD manifests as a wide spectrum of signs and symptoms. The classic form is characterized by positive CD serology; villous atrophy in the small intestine leading to malabsorption, chronic diarrhea; and weight loss, although these symptoms are not always present. CD is often associated with extra-intestinal comorbidities, such as osteoporosis, peripheral neuropathy, anemia, and infertility. If not diagnosed and treated, CD has been associated with the presence of malignancy, such as neoplasia in the gastrointestinal tract.[1-8]

It has been suggested that CD has oral manifestations, such as delay in dental eruption,[9] reduction of salivary flow, recurrent aphthous stomatitis,[9-12] angular cheilitis,[9,10] and dental enamel defects (DEDs) in both permanent[9-17] and primary dentitions.[9] To our knowledge, there are no studies evaluating oral manifestations in Brazilian patients with CD, which presents a unique genetic background.

The American Academy of Pediatric Gastroenterology, Hepatology, and Nutrition has reported that there is a high prevalence of DEDs in patients with CD, even

Statement of Clinical Relevance

This article highlights the importance of in recognizing the oral manifestations of celiac disease, which is a severe disease with a large number of asymptomatic carriers, and encouraging children with dental enamel defects, recurrent aphthous stomatitis, and reduction of salivary flow to be tested for celiac disease.

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[a]Professor, Department of Pediatric Dentistry, University of São Paulo – School of Dentistry of Ribeirão Preto, Ribeirão Preto, São Paulo, Brazil.
[b]Professor, Department of Pediatric Dentistry, University of São Paulo – School of Dentistry of Ribeirão Preto, Ribeirão Preto, São Paulo, Brazil.
[c]Professor, Department of Pediatric Dentistry, University of São Paulo – School of Dentistry of Ribeirão Preto, Ribeirão Preto, São Paulo, Brazil.
[d]Professor, Department of Childcare and Pediatrics, University of São Paulo – School of Medicine of Ribeirão Preto, Ribeirão Preto, São Paulo, Brazil.
[e]Professor, Department of Physics, University of São Paulo - School of Philosophy, Sciences and Letters of Ribeirão Preto, Ribeirão Preto, São Paulo, Brazil.
[f]Full Professor, Department of Pediatric Dentistry, University of São Paulo – School of Dentistry of Ribeirão Preto, Ribeirão Preto, São Paulo, Brazil.
[g]Full Professor, Department of Pediatric Dentistry, University of São Paulo – School of Dentistry of Ribeirão Preto, Ribeirão Preto, São Paulo, Brazil.

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in individuals with asymptomatic forms of the disease,[6] and recommends that individuals with DEDs with no obvious etiology should be referred for CD diagnosis. However, only a few studies have investigated the prevalence of CD in children with DEDs. Martelossi et al. revealed a 19.23% prevalence of CD in a group of Italians who presented with DEDs,[16] and in another study, CD was diagnosed in 17.86% of a sample of Egyptians patients with DEDs.[18] More studies are needed to confirm the hypothesis that children with DEDs could have undiagnosed CD. In addition, the chemical composition of dental enamel in CD has not yet been evaluated. Until now, only one scanning electron microscopic study[17] has assessed the structural aspects of DEDs in primary and permanent teeth of children with CD and those without CD. The study found that in individuals with the disease, the hypoplastic areas were highly hypomineralized, had shorter prisms, and presented more irregularly distributed and less interprismatic substance.[17]

Furthermore, until now, no studies have evaluated the oral clinical manifestations of CD in affected children, the chemical alterations in dental enamel, and the occurrence of CD in a sample of children with DEDs in a multilevel aspect. Thus, the aim of the present study was to evaluate in a sample of Brazilian children with CD (1) the oral manifestations (DEDs, RAS, and dental caries) and salivary parameters (pH, flow rate, and buffering capacity) in the children diagnosed with CD compared with control patients; (2) the chemical composition of the enamel of primary teeth in the children with CD and those without CD; (3) the occurrence of CD confirmed by clinical and serologic examinations in another sample of asymptomatic individuals with DEDs.

METHODS

The research protocol was approved by the Ethics Committee of the University of São Paulo of the School of Dentistry of Ribeirão Preto (Process 2010.1.1149.58) and the University Hospital of Ribeirão Preto, University of São Paulo, Medical School of Ribeirão Preto (Process 14026/2010).

Oral manifestations in children with or without celiac disease

The sample was composed of 52 children, aged 2 to 15 years, diagnosed with CD by the Pediatric Gastroenterology Service of the University Hospital of Ribeirão Preto, Medical School of Ribeirão Preto, University of São Paulo. The control group included 52 age- and gender-matched children with negative diagnosis and negative family history of CD and no gastrointestinal signs and symptoms of the disease, regularly attending the Pediatric Dentistry Clinic of the School of Dentistry of Ribeirão Preto, University of São Paulo. The exclusion criteria for both groups were presence of local causes associated with DEDs (i.e., history of trauma in the primary dentition); dental fluorosis or other systemic conditions, such as porphyria, congenital hemolytic anemia, chronic renal failure, and premature birth; and use of medications that could cause dental pigmentation, for example, tetracycline.

Table I. Classification of dental enamel defects in celiac disease according to Aine[13]

Grade 0: No defects.
Grade I: Defect in enamel color. Single or multiple cream-colored, yellow, or brown opacities with clearly defined or diffuse margins; in addition a part or the entire surface of enamel is without shiny surface.
Grade II: Slight structural defects. Enamel surface rough, filled with horizontal grooves or shallow pits; light opacities and discolorations may be found; in addition, a part or the entire surface of enamel is without shiny surface.
Grade III: Evident structural defects. A part or the entire surface of enamel rough and filled with deep horizontal grooves that vary in width or have large vertical pits; large opacities of different colors or strong discolorations may appear in combination.
Grade IV: Severe structural defects. The shape of the tooth has changed: The tips of cusps are sharp pointed and/or the incisal edges are unevenly thinned and rough; the thinning of the enamel material is easily detectable and the margins of the lesions are well defined; the lesion may be strongly discolored.

After anamnesis, a professional dental prophylaxis and oral examination were performed by a single pediatric dentist (FKC) previously calibrated to assess DEDs and dental caries, with a kappa intraexaminator concordance index score of greater than 0.80. DEDs were graded by using the Aine classification (grades I to IV) (Table I).[13] Both unspecific and systematic DEDs were recorded. Systematic DEDs had to be symmetric, that is, involving the same teeth in both the right and left hemiarches. DEDs were classified as nonspecific if they only affected the tooth in one hemi-arch and not in the other. Teeth were excluded from the assessment if more than two thirds of the coronal surface was restored, if there were large caries lesions, and if they were fractured.

The presence of aphthous stomatitis lesions at the time of the clinical evaluation was recorded. Self-recorded RAS history was also collected during the anamnesis.

The assessment of dental caries was performed by using a ball-ended probe and a clinical mirror under direct lighting, according to the World Health Organization criteria (1997),[19] and by using the DMFT (decayed, missed, or filled tooth) index.

The salivary parameters (pH, flow rate, and buffering capacity) were analyzed by using the Saliva-Check Buffer kit (GC America, GC America Inc., Alsip, IL).

Before saliva collection, the parents or patients were instructed to not perform oral hygiene, smoke, ingest drinks or foods within at least 1 hour of the procedure, and not use oral antiseptic mouthwashes within the previous 12 hours. The collection and evaluation of salivary samples occurred always in the morning, following the manufacturer's instructions on the kit.

Evaluation of the Chemical composition of primary teeth enamel

Sample collection and preparation. For the evaluation of the chemical composition of the primary dental enamel of individuals with or without the disease, exfoliated maxillary and mandibular caries-free primary molars without cracks, fractures, or shape abnormalities were obtained from the children with CD (n = 10 teeth) and controls (n = 10 teeth).

The teeth were cleaned of surface-adhered tissue remnants and debris with a hand scaler and rubber cup or pumice prophylaxis and were stored in a 0.1% thymol solution at 4 °C. At the moment of use, the teeth were rinsed in running tap water for 24 hours to eliminate traces of the preservative solution. Each tooth was bisected longitudinally in a mesiodistal direction to separate the crowns into two fragments: buccal and lingual or palatal. For each group, 10 fragments were randomly selected for analysis by energy dispersive x-ray spectroscopy (EDX) and 10 fragments for analysis by Fourier transform infrared spectroscopy (FTIR).

EDX analysis. The included fragments were flattened by using a 600-grit silicon carbide paper in a mechanical polisher under water cooling; hand polished with wet 1200-grit silicon carbide paper and 0.3-mm and 0.05-mm alumina paste on cloth; and cleaned in an ultrasonic bath for 10 minutes. The fragments were dehydrated in an ascending ethanol series (25% for 20 minutes; 50% for 20 minutes; 75% for 20 minutes; 90% for 30 minutes; 100% for 60 minutes), immersed in hexamethyldisilazane for 10 minutes for chemical drying, fixed on stubs, and sputter-coated with a gold layer. Microanalysis of the chemical composition of dental enamel was performed by using a high-resolution scanning electron microscope equipped with an x-ray detector system (Philips FEG, Philips, Eindhoven, The Netherlands) with a 5-nm spot size and operating at 20.0 kV. The equipment was connected to a computer system for data acquisition and processing. A quantitative analysis of the dental enamel was performed to detect concentrations of the elements oxygen (O), calcium (Ca), and phosphorus (P). In each specimen, two readings were obtained for each chemical element in two areas of 0.16 mm^2, each with a 60-second scanning time per area. Link ISIS Series 300 microanalysis system software (Oxford Instruments Ltd., UK) was used to obtain the mean values of O, Ca, and P concentrations as well as the Ca/P ratio.

FTIR analysis. The FTIR absorption spectra were recorded on a Nicolet-380 FT-IR Spectrometer (Nicolet, Vernon Hills, IL), using 32 scans for acquisition of each spectrum in the range of 4000 to 400 cm^{-1}, with maximum resolution of 0.5 cm^{-1}. The spectroscope was coupled to an accessory that allows for spectrum acquisitions between 4000 and 900 cm^{-1} in the attenuated total reflectance (ATR) mode.

The dental fragments were fixed in Plexiglass plates, and approximately 1 mg of enamel powder was collected from each specimen by grinding with a water-cooled high-speed diamond bur and placed on a 2-mm window of the diamond ATR accessory (DuraScope: Smiths Detection, Danbury, CT). The absorption spectra were uploaded by using Microcal Origin 8.0 graphing and data analysis software (Origin Lab Corp., Northampton, MA), and the phosphate absorption band at 1220 to 888 cm^{-1} and the carbonate absorption band at 1595 to 1300 cm^{-1} were measured in each sample. The band area of each chemical compound was delimited, the background was subtracted, and the area under each band was integrated using Microcal Origin 8.0 software tools. Each spectrum was normalized by the phosphate band area (1220−888 cm^{-1}), and the carbonate-to-phosphate ratio was calculated.

Evaluation of the occurrence of CD in children with DEDs

Another sample of 50 asymptomatic children, aged 2 to 12 years, with DEDs was included to investigate the occurrence of CD. The diagnosis was confirmed by clinical and serologic examination. The exclusion criteria were the same as those described previously.

The clinical examination was performed by the Pediatric Gastroenterology Service of the University Hospital of Ribeirão Preto, Medical School of Ribeirão Preto, University of São Paulo. For the serologic tests, 3 mL of blood was obtained from each child with DEDs. Serum samples were evaluated with respect to the presence of the antibody IgA anti-transglutaminase by using ELISA with the commercial kit Celikey Tissue Transglutaminase (human recombinant) IgA Antibody Assay IgA ELISA (Thermo Fisher Scientific, Uppsala, Sweden), according to the manufacturer's instructions. The following reference values of this kit were used: anti-tTG 10 less than IU = negative; anti-tTG between 7 and 10 = undetermined; and anti-tTG greater than 10 IU = positive.

Statistical analysis

The results were tabulated and compared by using the software SAS (Statistical Analysis System) for Windows version 9.1.3 (SAS Institute Inc., Cary, NC) and EpiInfo

Table II. Population characteristics

Variable	Children with celiac disease	Control subjects	P value
Gender – n (%)			
Male	18 (34.62)	23 (44.23)	.1627
Female	34 (65.38)	29 (55.77)	
Age – mean (SD)	11.59 (5.74)	11.48 (5.26)	.9129
Type of dentition – n (%)			
Primary	5 (9.61)	4 (7.69)	.8816
Mixed	32 (61.53)	31 (59.61)	
Permanent	15 (28.84)	17 (32.69)	
Salivary pH – mean (SD)	7.10 (0.35)	7.26 (0.30)	**.0165**
Salivary buffer – n (%)			
Low	6 (12.0)	10 (16.0)	.3577
Normal	44 (88.0)	40 (80.0)	
Salivary flow – n (%)			
Low	18 (36.0)	6 (12.0)	**.0060**
Normal	32 (64.0)	44 (88.0)	
Recurrent aphthous stomatitis – n (%)			
Yes	21 (40.38)	9 (17.31)	**.0052**
No	31 (59.61)	43 (82.69)	
DED – n (%)			
Yes	32 (61.54)	11 (21.15)	**.00001**
No	20 (38.46)	41 (78.85)	
DMFT – mean (SD)	2.11 (2.0)	3.90 (5.2)	**.0024**

DED, dental enamel defects; *DMFT*, decayed, missed, or filled tooth; *SD*, standard deviation.
Note: Bold font indicates statistically significant difference.

3.5.2. Data on categorical variables (DEDs, occurrence of recurrent aphthous stomatitis, pH, salivary flow, and buffering capacity of saliva) were analyzed by using the chi-square test. Data on continuous variables and normal distribution (FTIR and number of teeth with DEDs) were analyzed by using the *t* test and the Fisher exact test. In addition, the logistic regression analysis was implemented to evaluate the main outcome controlled by other covariates. Odds ratio was used to compare the relative odds of the occurrence of the outcomes in the children with CD. Data on non-normal distribution (DMFT and EDX) were analyzed by using the Wilcoxon test. The level of significance was set at 5% for all statistical analyses.

RESULTS

Oral manifestations

The mean ages were 11.59 years (SD = 5.74) for the children with CD and 11.48 years (SD = 5.26) for controls, and there was no statistically significant difference between the groups ($P = .9129$). Among the children with CD children, 65.38% were females and 34.62% were males; among the controls, 55.77% were females, and 44.23% were males. There was no significant difference between groups ($P = .1627$). The population characteristics and the comparison between the groups are presented in the Table II.

In 40.38% of the children with CD and in 17.31% of control children RAS was present, and this difference was statistically significant ($P = .0052$). In the children with CD, dental caries experience was lower than in the control group. DMFT mean values were 2.11 (SD = 3.2) and 3.90 (SD = 5.2) for the children with CD and those without CD, respectively ($P = .0024$).

With regard to the analysis of salivary parameters, 64% of the children with CD and 88% of controls had normal salivary flow. Low salivary flow was observed in 36% of the children with CD and 10% of the controls. Salivary flow was very low in only one (2%) of the children without CD. There was significant difference between the groups ($P = .0060$). The saliva of the majority of the children had normal or high pH (86% for those with CD and 92% for those without CD), and only few presented low pH (12% for those with CD and 8% for those without CD). Salivary pH was very low in only one of the children with CD. The salivary pH mean of the children with CD was 7.10 (SD = 0.35), whereas in the control group, it was 7.26 (SD = 0.30). There was significant difference between the two groups ($P = .0165$). The buffering capacity was low in 12% and normal in 88% of the children with CD. Among the controls, 4% had very low, 16% had low, and 80% had normal buffering capacity ($P = .3577$). The results of these parameters are also presented in the Table II.

The children with CD had DEDs in 61.54% of the cases (57.70% systematic defects and 3.84% unspecific DEDs), whereas in the control patients, only 21.15% had DEDs (13.46% systematic DEDs and 7.69% unspecific DEDs). By using stratified analysis adjusted by dentition (chi-square test), a statistically significant association ($P = .00001$) was observed between the specificity of DEDs and CD. DEDs pattern and distribution between the groups are presented in Table III. According to the Aine classification, grade I defects were observed in 44.24% of the children with CD (Figure 1) and 13.47% of controls; grade II defects were detected in 15.38% of the children with CD and 3.84% of controls. Grades III and IV defects were detected in 1.92% and 3.84% in the children with CD and controls, respectively (Figure 2). The DED grade difference between the groups was statistically significant ($P = .002$). With regard to the number of teeth with DEDs, 31.37% of the children with CD had 1 to 6 affected teeth compared with 15.39% of the controls. A total of 29.41% of the children with CD had more than 6 affected teeth compared with 5.77% of the controls. The mean number of teeth with DEDs was 7.37 among the children with CD and 4 among the controls. The number of affected teeth was associated with CD ($P < .0001$). Although canines were five times more affected by DEDs in the children with CD, statistically significant difference was seen only with regard to incisors and molars ($P < .005$).

Table III. Dental enamel defects: pattern and distribution

Enamel defects pattern	*Children with celiac disease*	*Control patients*	*Ratio*	*P value*
Group of affected teeth − n (%)				
Incisors	28 (53.8)	11 (21.2)	2.5:1	.0003
Canines	5 (9.6)	1 (1.9)	5:1	.1024
Premolars	2 (3.8)	1 (1.9)	2:1	.500
Molars	23 (44.2)	8 (15.4)	2.9:1	**.0007**
Enamel defect pattern − n (%)				
Hypomineralization	23 (44.24)	7 (13.47)		**.0002**
Mild hypoplasia	8 (15.38)	2 (3.84)		
Moderate hypoplasia	1 (1.92)	0 (0.0)		
Severe hypoplasia	0 (0.0)	2 (3.84)		
Number of affected teeth Dental enamel defects mean (SD)	4.5 (4.7)	0.8 (2.1)		**< .0000**

Note: Bold font indicates statistically significant difference.

Fig. 1. Grade I (Aine classification) dental enamel defects in an 8-year-old boy with celiac disease.

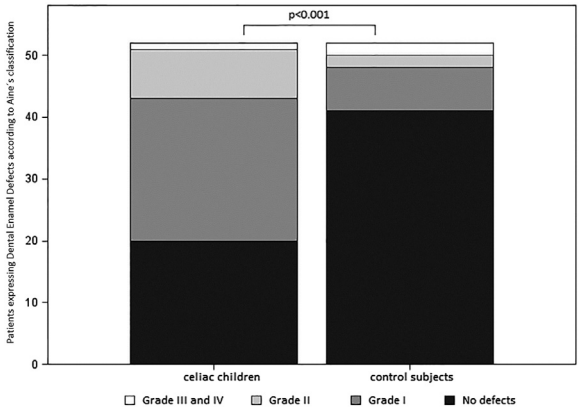

Fig. 2. Dental enamel defects according to the Aine classification in children with celiac disease and control patients, expressed in percentage ($P < .05$).

To control the interaction between some factors, a logistic regression analysis was performed. Table IV presents the results of the multivariate analysis, which demonstrated that CD is a protective factor for dental caries experience (OR = 0.74; 95% CI 0.61−0.91; $P = .0042$), whereas CD was a risk factor for RAS (OR = 3.23; 95% CI 1.30−8.01; $P = .0164$).

Chemical composition of enamel

In the EDX analysis (Table V), a significant difference between the groups in O, Ca, and P concentrations was not observed. However, a significantly lower Ca/P ratio ($P = .0136$) was observed in the primary dental enamel of the children with CD compared with controls. In the FTIR analysis, no significant difference was observed ($P = .5862$) in the mean values of carbonate-to-phosphate ratio in the primary dental enamel of the children with CD compared with controls.

Evaluation of the occurrence of CD in children with DEDs

On the basis of the results of the serologic tests, no subject with DEDs was considered reactive to the antibody IgA anti-transglutaminase.

DISCUSSION

In the present study, the higher occurrence of CD was seen among females (65.38%). This is in accordance with previous epidemiologic data that indicate an average female-to-male ratio of 2:1 in the prevalence of this condition.[2,20]

With regard to the presence of DEDs, a higher occurrence was observed in the children with CD compared with controls (61.54% and 21.15%, respectively; $P < .0001$), similar to the results of the majority of studies that evaluated DEDs in patients with CD.[10-16,21] Only three studies did not show a significant association between CD and DEDs. It is interesting that the authors of these studies attributed the absence of significant differences, in part, to the high occurrence of DEDs (38% to 68%) in CD-free individuals.[22-24] In the present study, a higher occurrence of systematic DEDs was found in patients with CD (57.70%) compared with controls (13.47%). These data are concordant with the results from previous studies, since the mean prevalence rates of systematic DEDs vary from 38% to 96%

Table IV. Multivariate analysis

Main outcome	*Covariants*[*]	*Z score*	*Odds ratio*	*Confidence interval (95%)*	*P value*
DMFT	DED, pH, salivary flow	−2.864	0.74	0.61−0.91	**.0042**
Recurrent aphthous stomatitis	pH	−2.398	3.23	1.30−8.01	**.0164**

Note: Bold font indicates statistically significant difference.
*Covariates used in the logistic regression analysis.

Table V. Oxygen (O), phosphorus (P), and calcium (Ca) concentration and Ca/P ratio in dental enamel of primary teeth of celiac children and control patients

Element	*Median*	*Minimum−Maximum*	*P value*
Oxygen			
Children with celiac disease (CD)	47.69	35.76−56.36	.8230
Control patients	50.21	26.01−57.29	
Phosphorus			
Children with CD	20.92	16.98−28.20	.1126
Control patients	18.90	17.05−24.02	
Calcium			
Children with CD	30.69	23.49−36.06	0.6553
Control patients	30.37	24.61−50.59	
Ca/P proportion			
Children with CD	1.35	1.20−2.08	0.0136[*]
Control patients	1.58	1.42−2.15	

*Significant difference.

in children with CD and from 0.6% to 17% in the general population.[14,16]

In the children with CD, the most frequent type of defect (44.24%) was hypomineralized enamel areas (Aine grade I), followed by enamel hypoplasia (17.3%; Aine grades II, III and IV). These results are similar to those of other studies that also detected a higher occurrence of grade I DEDs in patients with CD.[10,12,14,15]

The mean number of teeth with DEDs per subject was higher in the CD group, which can be explained by the higher occurrence of systematic defects in children with CD, as highlighted by other studies.[14,16,21] The pattern of DEDs was slightly different between the groups. Canine teeth were five times more affected by DEDs in the children with CD; however, only incisors and molars showed statistically significant difference. These results suggest that the presence of DEDs in patients with CD may have a temporal correlation with the time of diagnosis of the disease and the consequent cessation of gluten consumption. The period of the cessation of the immunologic reaction during amelogenesis could lead to different groups of tooth patterns in DEDs. This hypothesis needs to be verified by further studies.

In this study, a lower DMFT was found in the children with CD, which is consistent with the findings of previous studies.[12,14] In fact, the multivariate analysis demonstrated that CD acts as a protective factor in the caries experience. This result can be explained by the fact that these individuals should maintain a rigid diet free of gluten, which is a protein present in several cariogenic foods, such as oatmeal, flours, and breads, among others.[9,11]

Assessment of some salivary parameters in children with CD is important because of the possibility of an association between gluten intolerance and other autoimmune diseases that alter the saliva, such as type I diabetes mellitus[25] and Sjögren syndrome.[21] In the present study, salivary flow was significantly reduced in the children with CD, and 36% of them had a low salivary flow compared with 10% of the controls. These results are similar to those observed by Patinen et al. (2004), who found that 40% of CD children had low salivary flow not related to Sjögren syndrome.[21] The biochemical parameters of saliva (pH and buffering capacity) evaluated in the present study did not show significant differences between the children with CD and the controls, as reported elsewhere.[12,26]

The occurrence of RAS was significantly higher in the children with CD (40.38%) compared with the controls (17.31%), which corroborates the findings of previous studies.[10,11,24] In fact, our multivariate analysis showed CD to be a risk factor for RAS. Although the reason for the association between RAS and CD is still unknown, it has been speculated that the association may be the result of the autoimmune nature of these conditions.[12] Therefore, the presence of idiopathic aphthoid lesions, especially in individuals with DEDs, has been considered a relevant criterion for suspected CD.[11,12,24] In the present study, some of the children with CD who were on a gluten-free diet were observed to have aphthous ulcers. This is an interesting finding because it has been suggested that adherence to a gluten-free diet could reduce lesion recurrence,[10] but such an association was not observed in the present study or by Acar et al.[11]

To our knowledge, the present study was the first to analyze the chemical composition of the dental enamel of the primary teeth of patients with CD with the use of EDX and FTIR. These techniques are complementary, as EDX detects and quantifies the concentration of the chemical elements, and FTIR identifies and quantifies chemical compounds.[27] The tested hypothesis was that compared with control patients, children with CD have differences in dental enamel composition, even in the

absence of clinically detectable defects. No significant difference was found between the two groups for the chemical elements (O, P, and Ca) analyzed separately. However, the Ca/P ratio was significantly lower in the teeth of the children with CD. With regard to the stoichiometry of hydroxyapatite, the main component of dental enamel, the decrease in Ca/P ratio could be explained by the incorporation of carbonate in the tissue structure, which would increase its solubility.[27,28] However, FTIR spectroscopy did not reveal significant differences in the carbonate-to-phosphate ratio in dental enamel while comparing the two groups of children.

Previous studies have analyzed the chemical composition of the enamel of primary teeth[22] and the hypomineralized enamel of permanent teeth in healthy children.[27-29] However, comparison of our results with the literature is hindered by the fact that no other study has, so far, evaluated the chemical composition of dental enamel, specifically in patients with CD. In the present study, the Ca/P ratio was 1.35 for the children with CD and 1.58 for the controls ($P = .0136$), whereas in the studies cited above, the Ca/P ratio ranged between 1.5 and 2.1 (mean value of 1.8) for both primary and permanent teeth.[23,27-29] The Ca/P ratio recorded in the primary teeth of the patients with CD in the present study (1.35) is comparable with the value found in hypomineralized permanent teeth (1.4).[28]

Dental enamel is composed of 95 to 97 wt% carbonated hydroxyapatite with less than 1 wt% organic material. This tissue presents a dynamic exchange of ions with the environment because its own structural organization of apatite allows for the replacement and adsorption of ions in its composition. This dynamic becomes more intense in the most superficial layers of the enamel, where more ions exchange occur between the tooth and saliva.[27,28] Perhaps the differences in dental chemical composition of patients with CD can be better characterized in deeper enamel layers or in other mineralized dental tissues (i.e., dentin).

Further studies using different methods should be performed to evaluate not only the chemical differences but also the morphologic and physical differences between primary and permanent teeth with or without DEDs in patients with CD.

The hypothesis that asymptomatic patients who have DEDs could also have CD was also tested in the present study, but no confirmed diagnosis was achieved among the evaluated children. There are only two studies that have conducted a similar evaluation. The first one, conducted in an Italian population, found positive serology for CD in 10 of the 52 children who had DEDs, resulting in 19.23% of positive diagnosis[14]; the other study in an Egyptian population found similar results (17.86%).[18] Both studies showed a high prevalence, considering that the estimated worldwide prevalence of CD is approximately 1%. The lack of association found in the present study could be attributed, in part, to the small sample size or to the lower prevalence of CD in the Brazilian population.[20] Besides, this could be explained by the genetic background of the Brazilian population. Brazil is a large country with a highly mixed population consisting of indigenous Amerindians and immigrants from Europe, Africa, and Asia, and genetic differences can be related to the noncomparability of some oral diseases.[20,25,30] We also recommend further investigation of the prevalence of CD in individuals who exhibit one or more oral manifestations of the disease, such as RAS and systematic DEDs, with larger and more representative samples.

CONCLUSIONS

Brazilian children with a confirmed CD diagnosis had a higher rate of systematic DEDs and RAS, as well as lower DMFT and reduced salivary flow. The majority of the DEDs in the children with CD were found in the permanent incisors and molars, were of the systematic type, and classified as Aine grade I defects. A significant decrease of the Ca/P ratio was observed in the enamel of the primary teeth of the children with CD. In the studied population, no subject with DEDs was considered reactive to the antibody IgA anti-transglutaminase.

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Reprint requests:

Fabrício Kitazono de Carvalho, PhD
University of São Paulo - School of Dentistry of Ribeirão Preto
Avenida do Café S/N
14040-904 Ribeirão Preto
SP, Brazil
Fabricio@hotmail.com